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# カニクイザルにみられる摂餌量の低下を伴わない体重減少 －4および13週間反復投与毒性試験動物の比較－

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# Body weight loss without reduction in food consumption observed in cynomolgus monkeys –Comparison of animals in 4- and 13-week repeated dose toxicity studies–

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## Objective

Stress-related weight loss is occasionally observed in monkeys during non-clinical toxicity studies and can be challenging to differentiate from test article effects. At the 46th Annual Meeting of the Japanese Society of Toxicology, we investigated the incidence and physiological and pathological characteristics of control animals that lost 10% or more of their body weight during the toxicity study period and reported that the cause was related to stress and that improvements had been made through improvements in animal welfare. This study compared control data from a 4-week and a 13-week repeated dose toxicity study to examine differences in stress sensitivity and responsiveness due to varying study durations. We investigated the body weight trends and physiological and pathological characteristics of animals that lost 10% or more of their body weight in each study.

## Materials and Methods

**Animals**

Animals in the control groups of the 4-week and 13-week studies conducted at the test facility from 2010 to 2022 were retrospectively researched.

4-week study: 46 studies (male: n=206, female: n=196)

13-week study: 33 studies (male: n=146, female: n=136)

**Weight change rate**

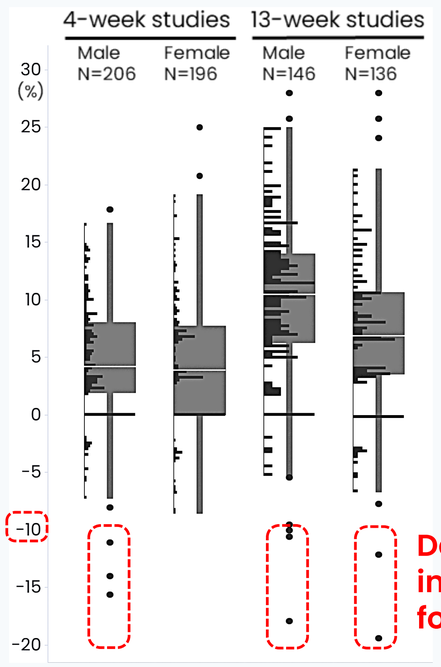
Weight change rate (%) was calculated as following formula.

(Final body weight – Initial body weight) × 100 / (Initial body weight)

**Other examinations**

Clinical observation, Food consumption, Hematology (CA-510, XT-2000iV), Blood chemistry (TBA-2000FR, TBA-200FR), Organ weight, Necropsy, and Histopathology

## Results




Detailed investigation follows

**Fig. 1. Weight change rate from the start of the acclimation period to the end of dosing period**

3 males (3/402 animals, 2/46 studies) in the 4-week studies and 3 males and 2 females (5/282 animals, 4/33 studies) in the 13-week studies showed a weight change rate of less than –10%.

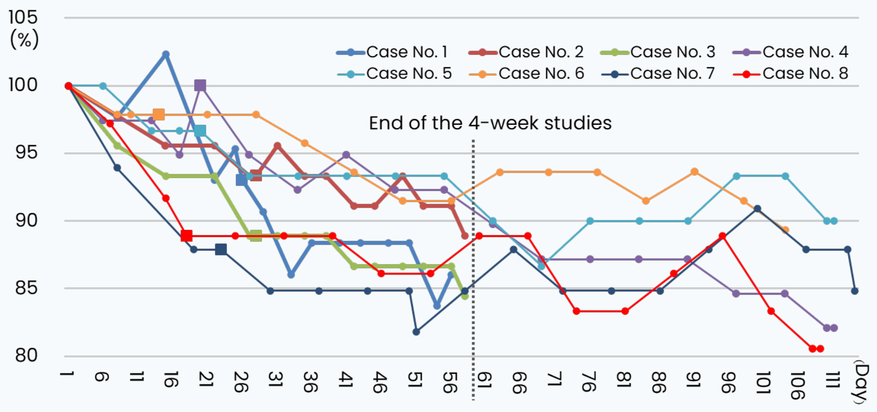



**Fig. 2. Weight transition in animals with weight loss**

■: Initial dosing day

Case Nos. 1-3: 4-week studies

Case Nos. 4-8: 13-week studies

The weight on the start day of acclimation set as 100%.

The horizontal axis shows the number of days from the start of acclimation, with the start day of acclimation set as Day 1.

Regardless of whether the study period was 4 weeks or 13 weeks, weight loss showed similar trend up to the end of the 4-week studies, i.e., rapid weight loss. After that, the weight changes plateaued or decreased slowly in the 13-week studies.

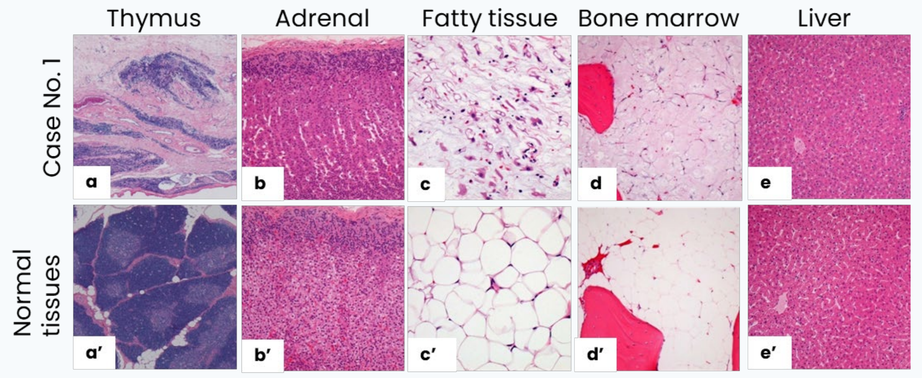



**Fig. 3. Histopathological changes of Case No. 1**

a: Atrophy

b: Hypertrophy of the zona fasciculata cells

c: Atrophy

d: Gelatinous atrophy

e: Decrease in glycogen in hepatocytes

These changes noted in the 4-week studies were similar to immunosuppressive changes caused by drug administration and secondary stress-related and wasting-related changes caused by administration in standard toxicity studies. On the other hand, there were no same changes in weight loss cases in the 13-week studies.

| Study design | 4-week studies | | | 13-week studies | | | | |
|---|---|---|---|---|---|---|---|---|
| Study No. | A | B | B | C | C | D | E | F |
| Case No. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
| Country of origin/sex/age | Vietnam/male/ 4 years old | Vietnam/male/ 6 years old | Vietnam/male/ 7 years old | China/male/ 4 years old | China/male/ 4 years old | Vietnam/male/ 4 years old | Vietnam/female/ 4 years old | Vietnam/female/ 4 years old |
| Number of days of rearing | 56 days | 58 days | 58 days | 110 days | 110 days | 104 days | 111 days | 109 days |
| Weight change | 4.3 kg to 3.7 kg | 4.5 kg to 4.0 kg | 4.5 kg to 3.8 kg | 3.9 kg to 3.2 kg | 3.0 kg to 2.7 kg | 4.7 kg to 4.2 kg | 3.3 kg to 2.8 kg | 3.6 kg to 2.9 kg |
| Weight change rate | –14.0% | –11.1% | –15.6% | –18.0% | –10.0% | –10.6% | –15.1% | –19.4% |
| Clinical sign | – | – | – | Crouching posture (Days 85-86) c) Diarrhea and loose stool (Days 81-92) | – | – | – | – |
| Food consumption a) | 100 g/100 g | 100 g/100 g | 96.5 g/100 g | 100 g/100 g | 95.5 g/100 g | 100 g/100 g | 97.7 g/100 g | 86.9 g/100 g |
| Hematology b) | Hemoglobin concentration↓(11.8 g/dL) Hematocrit↓(32.2 %) | – | Neutrophil↑ ($14.02\times10^3$/μL) | N.E. | N.E. | Red blood cell ↓ ($4.9\times10^6$/μL) Hemoglobin concentration↓ (12.5 g/dL) Hematocrit↓ (36.0%) | Neutrophil↑ ($25.26\times10^3$/μL) | Red blood cell ↓ ($4.49\times10^6$/μL) Hemoglobin concentration↓ (11.1 g/dL) Hematocrit↓ (35.9%) |
| Blood chemistry | Glucose↓(49 mg/dL) | Glucose↓(66 mg/dL) | – | N.E. | N.E. | Inorganic phosphorus↓ (2.6 mg/dL) | – | – |
| Organ weight | Thymus↓(0.63 g) | Thymus↓(0.28 g) | Thymus↓(0.80 g) | N.E. | N.E. | – | – | – |
| Necropsy | Thymus: Small | – | – | – | – | – | – | – |
| Histopathology d) | Thymus, Lymph nodes, Salivary glands: Atrophy Femur bone marrow: Gelatinous atrophy Sternal bone marrow: Gelatinous atrophy, Decrease in hematopoietic cells Fatty tissue: Atrophy e) Adrenal gland: Hypertrophy of the zona fasciculata cells Liver: Decrease in glycogen in hepatocytes | Thymus: Atrophy Femur bone marrow: Gelatinous atrophy Fatty tissue: Atrophy e) Pancreas: Atrophy | Thymus: Atrophy | – | – | – | – | – |

Clear differences from the 4-week studies

Table 1. Summary of various tests on monkeys with weight change rate of less than -10%

–: No noteworthy findings, ↑: High value, ↓: Low value

a) Mean daily food consumption/feeding amount. The weight loss occurred even though they could get enough food consumption.

b) Tendency of anemia was noted.

c) Hypoglycemia was suspected, and the animal was given a glucose injection, after which it showed improvement. The day of the initial dosing was designated as Day 1.

d) See Fig 3

e) Including visceral and subcutaneous fat

## Discussion

1. The reason stress-related histopathological changes were observed only in the 4-week study is presumably due to the acute stress experienced by the animals, resulting from a significant stress load and the onset of weight loss in a short period. In contrast, in the 13-week toxicity studies, the mild and sustained stress with gradual weight loss as chronic stress may not have been reflected in the histopathological changes.

2. There were low blood glucose levels in 2 cases in the 4-week toxicity studies, crouching posture suggesting hypoglycemia and low inorganic phosphorus levels in each 1 animal in the 13-week studies, and anemia tendencies in 1 case in 4-week study and 2 cases in 13-week studies. These results suggested that these monkeys were either unable to adequately absorb the nutrients they ingested, or they had an abnormality in their nutrient metabolism.

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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